

RRH: ACIDIFICATION IMPACTS ON FORAMINIFERA

LRH: MCINTYRE-WRESSNIG AND OTHERS

OCEAN ACIDIFICATION NOT LIKELY TO AFFECT THE SURVIVAL AND FITNESS OF
TWO TEMPERATE BENTHIC FORAMINIFERAL SPECIES: RESULTS FROM CULTURE
EXPERIMENTS

ANNA MCINTYRE-WRESSNIG, JOAN M. BERNHARD*, JOHANNES C. WIT, AND DANIEL C.

McCORKLE

Department of Geology and Geophysics, Woods Hole Oceanographic Institution, Woods
Hole, MA 02543, USA

* Correspondence author. E-mail: jbernhard@whoi.edu

ABSTRACT

Specimens of *Bolivina argentea* and *Bulimina marginata*, two widely distributed temperate benthic foraminiferal species, were cultured at constant temperature and controlled $p\text{CO}_2$ (ambient, 1000 ppmv, and 2000 ppmv) for six weeks to assess the effect of elevated atmospheric CO_2 concentrations on survival and fitness using Adenosine Triphosphate (ATP) analyses and on shell microfabric using high-resolution SEM and image analysis. To characterize the carbonate chemistry of the incubation seawater, total alkalinity and dissolved inorganic carbon were measured approximately every two weeks. Survival and fitness were not directly affected by elevated $p\text{CO}_2$ and the concomitant decrease in seawater pH and calcite saturation states (Ω_c), even when seawater was undersaturated with respect to calcite. These results differ from some previous observations that ocean acidification can cause a variety of effects on benthic foraminifera, including test dissolution, decreased growth, and mottling (loss of symbiont color in symbiont-bearing species), suggesting that the benthic foraminiferal response to ocean acidification may be species specific. If so, this implies that ocean acidification may lead to ecological winners and losers even within the same taxonomic group.

INTRODUCTION

Ocean acidification (OA) results from the uptake and dissociation of atmospheric carbon dioxide concentrations ($p\text{CO}_2$) into the world's oceans. Due to the steep increase in atmospheric CO_2 over the last 200 years (from ~280 ppmv to ~400 ppmv today), caused by human activities, today's open-ocean surface pH is ~0.1 unit lower than preindustrial values (Prentice et al., 2001; Feely et al., 2004; Royal Society, 2005; Hauri et al., 2009). If atmospheric $p\text{CO}_2$ reaches 800 ppmv by the end of this century (Prentice et al., 2001; Caldeira & Wickett, 2003; EPICA community members, 2004), ocean-surface pH could drop by another 0.3–0.4 units (Prentice et al., 2001; Orr et al., 2005; Feeley et al., 2009). The effects of such a pH decrease (and associated changes in other oceanic carbonate system parameters) on marine organisms, particularly those that produce calcium carbonate shells or skeletons, have been the focus of a wide range of recent studies.

Experimentally induced pH decreases (and the resulting decreases in the saturation states of calcite and aragonite; $\Omega_{\text{c/a}}$) lead to a reduction in calcification in many taxa (e.g., Gattuso et al., 1998; Langdon et al., 2000; Leclercq et al., 2000; Riebesell et al., 2000; Langdon et al., 2003; Shirayama & Thorton, 2005; Kuffner et al., 2008; Wood et al., 2008; Andersson et al., 2009; Clark et al., 2009; Comeau et al., 2009; de Moel et al., 2009; Maier et al., 2009; Martin & Gattuso, 2009; Moy et al., 2009; Semesi et al., 2009; Lombard et al., 2010; Pandolfi et al., 2011; Byrne et al., 2013). However, increases in calcification have also been observed (Iglesias-Rodriguez et al., 2008; Checkley et al., 2009; Ries et al., 2009; Beaufort et al., 2011; Lohbeck et al., 2012). Responses can even vary between subspecies (Langer et al., 2009; Lohbeck et al.,

2012). Other sub-lethal effects that have been observed in culture experiments using organisms with and without calcium carbonate skeletons or shells include reduced recruitment and growth rates, as well as changes in gene expression patterns, behavior, or physiology (Kuffner et al., 2008; Arnold et al., 2009; Ellis et al., 2009; O'Donnell et al., 2009; Parker et al., 2009; Walther et al., 2009; Dixson et al., 2010).

Foraminifera, among the most ubiquitous marine calcifying organisms (e.g., Langer et al., 1997; Small & Adey, 2001; Harney & Fletcher, 2003), are common to most marine habitats (Hemleben et al., 1989; Lee & Anderson, 1991; Sen Gupta, 1999; Murray, 2006). Their role in a future high $p\text{CO}_2$ ocean is of particular interest, since they constitute an important link in marine food webs (e.g., Legendre & Le Fèvre, 1995; van Oevelen et al., 2006) and play important roles in the recycling of organic carbon (e.g., Moodley et al., 2000) and the production of carbonate (Langer et al., 1997). In previous studies, where changing $p\text{CO}_2$ altered seawater carbonate chemistry, benthic foraminifera exhibited a range of responses. In some cases, calcification/growth continued at increased $p\text{CO}_2$ (Dissard et al., 2010; Fujita et al., 2011; Haynert et al., 2011; McIntyre-Wressnig et al., 2013). However, at very high $p\text{CO}_2$ (levels of $p\text{CO}_2$ differed between studies), calcification decreased in most species such as the temperate species *Ammonia tepida* [Cushman] (Dissard et al., 2010), *Ammonia aomoriensis* [Asano] (Haynert et al., 2011), and *Ammonia* sp. (Keul et al., 2013). The hyaline symbiont-bearing reef species *Baculogypsina sphaerulata* [Parker & Jones] and *Calcarina gaudichaudii* [d'Orbigny in Ehrenberg] showed increased calcification at intermediate $p\text{CO}_2$ levels, but net calcification in *Amphisorus hemprichii* [Ehrenberg], which secretes a porcelaneous shell, decreased at all elevated $p\text{CO}_2$ (Fujita et al., 2011). Moreover, in these symbiont-bearing foraminifera, calcification responses differed between two clonal populations of the same species and between

species with different test composition (Fujita et al., 2011). Test dissolution was not a general trend—no test dissolution was observed in the studies by Dissard et al. (2010), Fujita et al. (2011), and Keul et al. (2013 and references therein)—even in seawater undersaturated with respect to calcite. In contrast, the hyaline symbiont-bearing reef species *Amphistegina gibbosa* [d’Orbigny] exhibited test dissolution even in saturated conditions (McIntyre-Wressnig et al., 2013), and Haynert et al. (2011) observed test dissolution in *A. aomoriensis* in both saturated and undersaturated conditions. Survival, however, was not affected by elevated $p\text{CO}_2$ in these two studies. In summary, calcareous foraminifera (both hyaline and porcelaneous) display a number of species-specific responses to OA, ranging from dissolution to increased calcification and everything in between (Keul et al., 2013, and references therein).

In short-term (10–14 days) laboratory experiments, Bernhard et al. (2009a) showed that thecate benthic foraminifera survived and, in some cases, reproduced in $p\text{CO}_2$ up to 200,000 ppmv. During a month-long field experiment (Bernhard et al., 2009b), thecate and agglutinated foraminifera were not significantly affected by the release of liquid CO_2 on the deep-sea floor, which led to a decrease in bottom-water pH of up to 0.25 pH units. The survival of calcareous benthic foraminifera, however, was negatively impacted. At a naturally acidified field site, Dias et al. (2010) found no calcareous foraminifera below pH 7.6.

The aim of this study was to test the effect of elevated $p\text{CO}_2$ on two species of temperate benthic foraminifera: *Bulimina marginata* [d’Orbigny] and *Bolivina argentea* [Cushman]. *Bulimina marginata* was selected because it has been successfully cultured in the laboratory and has been the subject of geochemical paleoceanographic proxy calibrations (e.g., Hintz et al., 2006; McCorkle et al., 2008; Barras et al., 2010; Filipsson et al., 2010; Wit et al., 2012; Groeneveld and Filipsson, 2013). *Bolivina argentea* was selected because it is particularly

abundant in certain bathyal sites (Bernhard et al., 1997) and has recently been found to denitrify in regions of low oxygen (Bernhard et al., 2012). Experiments were conducted at ambient (~420 ppmv) and projected (1000 and 2000 ppmv) $p\text{CO}_2$ levels likely to occur in shallow-marine pore waters in the decades ahead, based on projected atmospheric $p\text{CO}_2$ values (Prentice et al., 2001; EPICA community members, 2004) and published $p\text{CO}_2$ data for similar habitats (Cai et al., 1995; Zhu et al., 2006).

MATERIAL AND METHODS

SPECIMEN COLLECTION, CULTURING, AND PREPARATION

Foraminifera-bearing sediments were collected on cruises during June 2008 and June 2009, using a Soutar box corer. Sediments for *Bol. argentea* (Fig. 1A) were collected from the northeast flank of the Santa Barbara Basin (~430-m water depth, 34°18.57'N, 119°54.18'W; Bernhard et al., 1997). Living specimens of *Bul. marginata* (Fig. 1B) were obtained from sediments at a site on the continental shelf, ~50 nmi south of Martha's Vineyard (the "Mud Patch", ~80-m water depth; centered on 40°30'N, 70°45'W; Bothner et al., 1981). For details of sediment collection and handling, see Hintz et al. (2006) and Filipsson et al. (2010). In the shore-based cold room, sediment samples were kept in a recirculating seawater system, designed to maintain stock cultures of benthic foraminifera (Chandler & Green, 1996). They were fed weekly with a concentrated algal mixture of *Dunaliella tertiolecta* and *Isochrysis galbana* (Hintz et al., 2004). To ensure that all individuals were alive at the start of each experiment, subsamples were taken from the sediment two days prior to the start of each experiment and incubated in

CellTracker™ Green CMFDA (CTG; Life Technologies) according to standard protocol (Bernhard et al., 2006). This fluorogenic probe (CTG) labels only living cells and has been used successfully to distinguish live from dead foraminifera (e.g., Bernhard et al., 2009b). After the incubation, sediments were sieved over a 90- μm screen using chilled seawater. The >90- μm fractions were then examined using a Leica MZ FLIII epifluorescence stereomicroscope equipped with optics for fluorescein detection. Specimens of all available sizes that fluoresced brightly in most of their chambers were picked using a small brush or pipette, and distributed randomly among experimental treatments.

EXPERIMENTAL OVERVIEW

For each species, experiments were run consecutively, at two “Elevated” atmospheric CO_2 concentrations, 1000 ppmv and 2000 ppmv, with ambient laboratory $p\text{CO}_2$ (~420 ppmv) as the “Control” concentration in each of the experiments (McIntyre-Wressnig et al., 2013). These levels were chosen because the Mud Patch sampling site is similar to sites in nearby Long Island Sound where pore-water $p\text{CO}_2$ is typically between ~800–5000 ppmv in the surface 1–2 cm (Zhu et al., 2006). In each experiment, only one species (either *Bol. argentea* or *Bul. marginata*) was used. Experiments lasted for 6 weeks, with intermediate foraminiferal analyses after 1, 2, and 6 weeks. Specimens were fed weekly during each experiment. Five experiments were conducted: three using *Bol. argentea* (two at 1000 ppmv, starting in November 2008 and September 2009, and one at 2000 ppmv, starting in October 2008) and two using *Bul. marginata* (at 1000 ppmv, starting in January 2009, and 2000 ppmv, starting in March 2009). Due to the static conditions in which the foraminifera were kept, excretion products and decomposing algae could have led to a

buildup of metabolic byproducts such as ammonia within the experiment treatment containers, potentially causing an increase in mortality in the experimental populations (e.g., Lin & Chen, 2003). However, such an increase would have occurred uniformly across all treatments.

INCUBATORS AND TREATMENTS

A feedback-controlled, infrared CO₂ sensor (Biospherix ProCO₂ system; accuracy of \pm 2% of target value) was used to maintain Elevated $p\text{CO}_2$ conditions. The sensor was mounted inside a Biospherix C-chamber that was housed in a water-jacketed darkened CO₂ incubator (Nuaire US Autoflow NU4950) attached to a refrigerated bath (Thermo RTE740) to maintain temperature at 7–8°C for the duration of each experiment (6 weeks; Fig. 2). This temperature is similar to the in situ temperatures at both collection sites, as determined by CTD casts during sediment collection. During the 2008 cruise, we measured the total alkalinity (T_A) and total dissolved inorganic carbon (T_C) of bottom water at the Mud Patch sampling site, and from these data calculated in situ pH_T and Ω_c values of 8.06 and 2.86, respectively, with CO₂SYS (Lewis & Wallace, 1998). A QuBit infrared CO₂ analyzer (Qubit Systems, Inc.) was used to calibrate the CO₂ sensor (N₂ gas blank and a 1036 ppmv CO₂ in N₂ gas standard). A pilot experiment was conducted to establish the length of time necessary for the seawater to equilibrate with the elevated $p\text{CO}_2$ atmosphere: spectrophotometric pH measurements using an Ocean Optics USB4000 spectrophotometer with a blue-filtered tungsten light source (Ocean Optics LS-1-LL) established that the $p\text{CO}_2$ of the seawater media equilibrated with the incubator atmosphere within 40 h (Fig. 3). Since the height of the spectrophotometer cuvettes was several times the depth of the water in our experimental Petri dishes and equilibration time is a function of the

square of the diffusion length, the seawater in the Petri dishes equilibrated in no more than a few hours. Dishes for the Control treatment were kept in a plastic desiccator (Nalgene), which had been covered with black electrical tape, in a 7°C cold room. This “Control” box was aerated daily by opening and closing the door repeatedly for ~40 seconds. The C-chamber and control box each also contained 15 Petri dishes with distilled water (DI) to ensure high humidity levels and minimized evaporation from the experimental dishes. When necessary, DI water in these Petri dishes was replaced. In the tables and figures, the controls are denoted by the $p\text{CO}_2$ of the associated treatment so that, for example, the “1000-ppmv control” is the control (400 ppmv) sample concurrently run during the 1000-ppmv experiment for that species. Treatment containers were Petri dishes (100-mm diameter), each containing 17 mL of seawater. Each Petri dish contained between 28–30 individuals. Once a week, all Petri dishes were moved within the incubator and control box randomly to avoid any location-specific effects. At each time point, all 28–30 specimens from one of the elevated treatment duplicates were extracted for adenosine triphosphate (ATP; Bernhard et al., 2009a) directly after removal from the elevated $p\text{CO}_2$ incubator—we refer to these as the “Direct” samples. Specimens from the second set of elevated $p\text{CO}_2$ treatments were moved to the control chamber after the enriched CO_2 incubation, where the seawater in the Petri dishes re-equilibrated with atmospheric (control) $p\text{CO}_2$ for ~24 h. After this time period, all specimens from these “Rebound” samples were also extracted for ATP. The Rebound treatment tested whether the physiological state under elevated $p\text{CO}_2$ can be reversed. Such a reversal has been observed in a thecate foraminiferal species after exposure to extremely high $p\text{CO}_2$ over a shorter time span (200,000 ppmv; Bernhard et al., 2009a). Only specimens from the Control and Direct treatments were used for test microstructure analysis.

CARBONATE SYSTEM PARAMETERS

The seawater used for these experiments was surface seawater collected from ~5-m depth using the uncontaminated seawater intake of an underway research vessel. This surface water was pumped into and stored in a 55-gallon polyethylene drum. For all but one experiment, the seawater collection was made in June 2008 at approximately 38°N, 69°15'W. For one of the replicate 1000-ppmv experiments using *Bol. argentea*, the seawater collection was made in September 2009 at ~39°N, 69°21'W. The foraminifera-bearing Petri dishes did not contain enough seawater for the T_A and T_C analyses, so uncapped 22-mL glass scintillation vials of seawater were kept in the Control chamber and the Elevated CO₂ incubators. During each experiment, vials were taken at four time points (at the end of weeks 1, 2, 4, and 6) for these chemistry analyses. After poisoning with 10 μ l HgCl₂ at the time of collection, alkalinity samples were stored in glass vials, and T_C samples were sealed in glass ampoules. As described in McIntyre-Wressnig et al. (2013), total alkalinity values were determined by automated Gran titrations of 1-mL samples, standardized using certified reference materials obtained from Dr. A. Dickson (Scripps Institution of Oceanography). The relative standard deviation of alkalinity analyses of replicate samples through all experiments averaged $\pm 0.9\%$ ($n = 39$). T_C values were determined manometrically on 3–5-ml samples, using an automated vacuum extraction system. The relative standard deviation of T_C analyses of replicate samples through the experiments averaged $\pm 1.1\%$ ($n = 47$). Calculations of carbonate system parameters (pH and calcite saturation state, Ω_c) were performed with CO2SYS (Lewis & Wallace, 1998), using the total pH scale and the dissociation constants of Hansson (1973 a, b) and Mehrbach (1973), as refit by Dickson & Millero (1987).

SURVIVAL ANALYSIS

The ATP content of individual foraminifera was used as an indicator of cellular energy to determine survival rates and individual fitness (e.g., DeLaca, 1986; Bernhard & Reimers, 1991; Bernhard, 1992; Bernhard et al., 2008). Due to the time-sensitive nature of ATP response and to minimize re-equilibration of the seawater in the Direct dishes, care was taken to start processing individuals within 5–10 min after removing the Petri dish from the incubator. After measuring (length, L, and width, W; Nikon SMZ-2B stereo dissecting microscope), individuals were extracted for ATP in 1.0-mL boiling phosphate-citrate buffer (DeLaca, 1986) for 5 min, and the extracts were then frozen for later analysis. Analysis of ATP content of thawed samples was performed on a Berthold Lumat LB 9507 luminometer and luciferin-luciferase reaction per standard protocol (e.g., Bernhard, 1992). In 10 specimens of *Bol. argentea*, width (W) and height (H) of the test was measured, and a W/H ratio calculated. This ratio was subsequently used to estimate H for all *Bol. argentea* specimens used in the ATP analysis. Test volume was used to normalize ATP data. Volumes were calculated as follows:

$$V_{\text{Bol. argentea}} (\text{flattened prolate spheroid}) = \pi/8(W/H)^2L \text{ and}$$

$$V_{\text{Bul. marginata}} (\text{cone}) = \pi/3(W/2)^2L.$$

A live-dead threshold of 415 ng mm⁻³ (Bernhard, 1992) was used for both species to determine if specimens were living at the time of ATP extraction.

TEST MICROFABRIC ASSESSMENT

Specimens intended for SEM analysis were rinsed in DI water and, air dried on micropaleontology slides, sputter-coated (Leica EM MED020; 10 nm palladium) and imaged using a Zeiss Supra 40VP Scanning Electron Microscope (McIntyre-Wressnig et al., 2013). For analysis, only the final few chambers of each test were used, because we estimate that the older parts of each test had been precipitated well before the start of the experiments.

RESULTS

CARBONATE SYSTEM PARAMETERS

The Ω_c and pH decreased with increasing $p\text{CO}_2$, but the seawater remained saturated with respect to calcite in all but one experimental treatment (Tables 1 and 2; Fig. 4). In the 2000-ppmv $p\text{CO}_2$ experiment using *Bul. marginata*, Ω_c dropped to 0.84 in the elevated treatments (Direct and Rebound; Table 2). To assess the possibility of significant changes in T_A due to foraminiferal calcification during the experiments, an estimate of mean growth was calculated using growth estimates from McCorkle et al. (2008) and the mean dry weight of specimens from bulk culture ($n = 9$). Bulk culture specimens had to be used because all specimens from the experiments were sacrificed for SEM analysis. We calculated ΔT_A as:

$$\Delta T_A = \mu\text{g CaCO}_3 * (1 \mu\text{mol CaCO}_3 / 100 \mu\text{g CaCO}_3) * (2 \mu\text{-equivalent Alkalinity} / 1 \mu\text{mol CaCO}_3).$$

The mean decrease in calculated T_A after six weeks due to calcification was negligible, amounting to 0.2% in the *Bul. marginata* experiments. No comparable growth rates are available for *Bol. argentea*, which is larger than *Bul. marginata* and slightly more heavily calcified (thicker walls). Even if the amount of CaCO_3 produced by *Bol. argentea* after six weeks was twice that of *Bul. marginata*, calcification would have led to a decrease in T_A of only ~0.3% in the *Bol. argentea* experiments. The Petri dishes housing the foraminifera were shallow and had a large surface area, so we assume that equilibration with the overlying gas phase set the T_C in the cultures.

BOLIVINA ARGENTEA

In the 1000-ppmv $p\text{CO}_2$ experiments on *Bol. argentea*, there were no significant differences in mean survival between the three treatments at any of the three time points ($p > 0.1$, ANOVA; Fig. 5). Furthermore, there was no significant difference in survival between all six treatments (1000-ppm $p\text{CO}_2$ Control, Direct, Rebound; 2000-ppmv $p\text{CO}_2$ Control, Direct, Rebound) after 6 weeks ($p > 0.1$, χ^2 -test). In the 1000-ppmv experiments, increased $p\text{CO}_2$ did not affect the fitness (i.e., ATP concentration) of survivors—there were no significant differences in mean ATP concentration of all three treatments after 1 and 2 weeks, and between Control and Rebound after 6 weeks ($p > 0.1$, ANOVA). No replicate numbers are available for Direct after 6 weeks due to lack of survival in one of the experiments, in which survival was very low in all three treatments. In the 1000-ppmv $p\text{CO}_2$ experiment, survival decreased more strongly in the elevated treatment than in the Control (Fig. 5), but this difference was not statistically significant. There was no significant difference in survival between treatment and control in the

2000-ppmv experiment (Fig. 5). Mean ATP concentration was similar in all three experiments, ranging from 3695–10046 ng mm⁻³, with most values falling into the 5000–7000-ng mm⁻³ range. The ATP concentration of survivors showed no pattern in any of the experiments. No differences in test microstructure could be detected between Control and Direct treatments (Fig. 6), indicating that there was no effect of increased *p*CO₂ (1000 ppmv and 2000 ppmv) on test microstructure of *Bol. argentea*.

BULIMINA MARGINATA

There was no significant difference in survival of *Bul. marginata* between all six treatments (1000-ppmv *p*CO₂ Control, Direct, Rebound; 2000-ppmv *p*CO₂ Control, Direct, Rebound) after 6 weeks ($p > 0.1$, χ^2 -test; Fig. 7). The mean ATP concentration was similar in both experiments, ranging from 1853–3948 ng mm⁻³. Over time, the ATP concentration of survivors increased in both the 1000 and 2000-ppmv *p*CO₂ Direct and Rebound treatments (Fig. 7), suggesting that fitness was not affected by elevated *p*CO₂. No differences in test microstructure could be detected between Control and Direct treatments (Fig. 8), indicating that there was no effect of increased *p*CO₂ (1000 ppmv and 2000 ppmv) on test microstructure of *Bul. marginata*.

DISCUSSION

No significant effects of elevated *p*CO₂ on survival and fitness were observed for either of the two studied species. This may be due to the fact that both species already experience high

and/or fluctuating $p\text{CO}_2$ in their natural habitat—sediments in the top few cm of the seafloor sediment column (Haynert et al., 2011, 2012). The pore waters just below the sediment-water interface typically have elevated CO_2 concentrations and lowered pH as a result of microbial organic-matter decomposition in the sediments (e.g., Emerson & Bender, 1981; Cai et al., 1995), and the foraminifera that inhabit these environments may be adapted to dealing with high $p\text{CO}_2$. A possible explanation for the slight yet statistically insignificant increase in ATP over time for all treatments is bacterial growth on or in the foraminiferal tests, although there was no evidence in our SEM investigations of such prokaryotic blooms on exterior test surfaces.

A similar pattern in survival rates has been observed in the benthic symbiont-bearing foraminifer *Amphistegina gibbosa*, which was exposed to the same $p\text{CO}_2$ conditions over the same time period (McIntyre-Wressnig et al., 2013), and high survival rates in response to similar experimental conditions have also been noted for other temperate species (Dissard et al., 2010; Haynert et al., 2011; Keul et al., 2013), although these rates were not quantified and survival was determined by methods other than ATP analysis. Despite the finding that a range of benthic foraminiferal species can survive in seawater with pH values well below 8.0 (as low as 7.38; Dissard et al., 2010; Fujita et al., 2011; Haynert et al., 2011; McIntyre-Wressnig et al., 2013), no calcareous foraminiferal species were found by Dias et al. (2010) in sediments beneath bottom water with a pH = 7.6, at a naturally acidified shallow (2-m) site in the Mediterranean Sea. It is not clear whether the observations made in the Dias et al. (2010) field study were the result of a long-term effect of OA or if they reflected some other process that was correlated with pH. Experimental results from an acidification study on *Ammonia beccarii* [Linnaeus] furthermore showed that pseudopodial activity was reduced at a pH of 7.5, while, at pH 7.0, all specimens started to decalcify (Le Cadre et al., 2003).

The lack of an OA effect on fitness observed in this study has also been shown for *Amphistegina gibbosa*, exposed to similar $p\text{CO}_2$ levels over six weeks in experiments by McIntyre-Wressnig et al. (2013). This observation was unexpected since specimens from the Elevated (Direct + Rebound) treatments would likely spend more energy on calcification than Control specimens. In hyaline foraminifera, such as the species studied here, carbon used for calcification comes from a large inorganic carbon pool, within intracellular vacuoles of seawater (ter Kuile et al., 1989; de Nooijer et al., 2008, 2009; Bentov et al., 2009). During transport to the calcification site, the pH of the seawater in these vacuoles increases by proton pumping (~ 0.5 – 1.0 units). This in turn leads to an increase in carbonate ion concentration $[\text{CO}_3^{2-}]$, because the relative CO_3^{2-} proportion of the total inorganic carbon in the endocytosed seawater increases and because aqueous CO_2 (present in the cytosol) diffuses across the gradient from the relatively acidic cytoplasm (pH 7.2–7.5) into the alkaline vacuoles. The lower the initial pH of the vacuolated seawater, the more energy has to be expended by the hyaline foraminifera to reach the required pH for calcification (Bentov et al., 2009). Because the pH (and Ω_c) of the seawater in our Elevated $p\text{CO}_2$ conditions was lower than in the Control conditions (and, noting, in the *Bul. marginata* 2000-ppmv experiments, that the seawater was undersaturated with calcite), it is surprising that fitness levels did not differ between these conditions. However, whether the fitness of both species remains unaffected when exposed to elevated $p\text{CO}_2$ for an entire life cycle remains to be determined.

Although no present-day pore-water data are available for the Mud Patch sediments, where the *Bul. marginata* were collected, that area has relatively high organic content ($\sim 2\%$; Bothner et al., 1981). It is unlikely that the Mud Patch sediments are as organic carbon-rich and seasonally depleted in oxygen as in Long Island Sound (Zhu et al., 2006), so present-day pore-

water $p\text{CO}_2$ is likely to be below 2000 ppmv but higher than bottom-water $p\text{CO}_2$. Sediments at the collection site in the Santa Barbara Basin, where *Bol. argentea* are very abundant in the top cm of the sediment, are also organic rich (Kuwabara et al., 1999), and are likely to have elevated $p\text{CO}_2$ and lowered pH and Ω values (Cai et al., 1995). The capability of *Bol. argentea* to denitrify (Bernhard et al., 2012) enables the species to live in low oxygen or even anoxic environments. Since low-oxygen marine settings are also typically characterized by elevated $p\text{CO}_2$ and low pH (Pörtner et al., 2005), it is probable that *Bol. argentea* is insensitive to such conditions, at least for the duration of our experiments.

Like survival and fitness, the test microstructures of these species were unaffected by our elevated $p\text{CO}_2$ treatments, even in the treatments where the seawater was undersaturated with calcite. Results from previous studies on benthic foraminifera suggest that effects of OA on test microstructure vary between species: in the temperate species *Ammonia tepida*, OA did not result in any test dissolution, even in seawater undersaturated with calcite ($\Omega = 0.5$; Dissard et al., 2010). Similarly, Fujita et al. (2011) did not observe any dissolution on the tests of three reef species with tests composed of high-magnesium calcite. On the other hand, Haynert et al. (2011) reported test dissolution in specimens of the temperate species *Ammonia aomoriensis* exposed to seawater supersaturated with calcite ($\Omega = 1.4$). Likewise, McIntyre-Wressnig et al. (2013) observed dissolution features on the tests of the symbiont-bearing benthic coral-reef foraminifer *Amphistegina gibbosa* in seawater at an even higher calcite saturation ($\Omega = 2.8$), and the fraction of the affected test area increased with decreasing Ω_c . An attribute of all foraminifera is their networks of branched and anastomosed reticulopods that can extend over the foraminiferan test surface (Travis & Bowser, 1991). In our experiments, reticulopods may have protected the test surface completely or partially from direct contact with the surrounding seawater, thereby

inhibiting test dissolution of our high $p\text{CO}_2$ specimens. Foraminiferal reticulopodial networks may thus have the same function as the protective organic layers that metazoans rely on to avoid dissolution at lowered carbonate saturation states, even though some can up-regulate calcification (Rodolfo-Metalpa et al., 2011).

The reasons that particular foraminiferal species may be better adapted to OA than others are a matter for debate. Our experiment was not designed to identify the adaptations to OA invoked by foraminifera. In invertebrate taxa, some increase calcification rates during pH stress, which results in a decrease in energy available for reproduction. From here, the key question for foraminifera is: Will longer-term OA exposure significantly impact reproductive rate and juvenile survival?

CONCLUSIONS

The present study, combined with results from previous OA culture studies using benthic foraminifera, indicates that there are species-specific responses to OA in benthic foraminifera and that the vulnerability of different species may be dependent on the initial conditions of their natural environment. Such species-specific responses have also been demonstrated in other taxa, such as coccolithophores (e.g., reviewed in Krug et al., 2011). Altogether, observations strongly imply that ecological winners and losers will emerge from within the foraminifera in response to OA. A more thorough understanding of foraminiferal response to OA must await lengthy investigations of full foraminiferal life cycles.

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TABLE CAPTIONS

TABLE 1: Salinity and carbonate chemistry of seawater during 6-week experiments using *Bol. argentea*. Values in brackets are standard deviations. Measurements were made after weeks 1, 2, 4, and 6. Note that two separate experiments were conducted at 1000-ppmv $p\text{CO}_2$, using two different batches of natural seawater.

TABLE 2: Salinity and carbonate chemistry of seawater during 6-week experiments using *Bul. marginata*. Measurements were made after weeks 1, 2, 4, and 6. Values in brackets are standard deviations.

FIGURE CAPTIONS

FIGURE 1. SEM micrographs of **A** *Bolivina argentea* and **B** *Bulimina marginata*. Scale bars = 100 μm .

FIGURE 2. Schematic of experimental setup for enriched $p\text{CO}_2$ treatments.

FIGURE 3. Decrease in pH in seawater exposed to 1000-ppmv $p\text{CO}_2$, measured using spectrophotometric pH analysis. Data points at 19 h (circled) indicate when incubator was opened for 10 min.

FIGURE 4. Mean calculated pH_T and Ω_c in five experiments (three using *Bol. argentea*, two using *Bul. marginata*) in which seawater was exposed to Control (420 ppmv) and 1000-ppmv or Control and 2000-ppmv pCO_2 , respectively. Note that Control values are averages from three *Bol. argentea* experiments and two *Bul. marginata* experiments, respectively, and that *Bol. argentea* 1000-ppmv values are averages from two repeat experiments. Error bars represent standard deviations.

FIGURE 5: Percent survival and mean ATP concentration of surviving *Bol. argentea* in two experiments using Control and 1000-ppmv (left panels), and one experiment using Control and 2000-ppmv elevated pCO_2 (right panels). Note that mean ATP content of the Direct treatment is not shown for 1000 ppm at week 6 due to the lack of survivors in one of the replicate experiments. Error bars represent standard error. No significant differences in survivorship and ATP content between treatments and time points were detected.

FIGURE 6. SEM micrographs of *Bol. argentea* from experiments using Control and 2000-ppmv elevated pCO_2 showing final (youngest) few chambers and aperture. Scale bars = 20 μm .

FIGURE 7. Percent survival and mean ATP concentration of surviving *Bul. marginata* in experiments using Control and 1000-ppmv (left panels) and Control and 2000-ppmv elevated pCO_2 (right panels). Error bars represent standard error. No significant differences in survivorship and ATP content between treatments and time points were detected.

FIGURE 8. SEM micrographs of *Bul. marginata* from experiments using Control and 2000-ppmv elevated $p\text{CO}_2$ showing the final (youngest) few chambers and aperture. Scale bars = 20 μm .

Seawater Type	Salinity	T _A	T _C	pH _T	Ω _{calcite}
		(μMol kg SW ⁻¹)			
1000-ppmv incubator	36.4 (0.3)	2396 (31)	2358 (36)	7.76 (0.1)	1.5 (0.3)
1000-ppmv control	36.4 (0.3)	2403 (26)	2279 (27)	8.01 (0.01)	2.4 (0.5)
2000-ppmv incubator	36.6	2470 (96)	2495 (84)	7.60 (0.1)	1.1 (0.2)
2000-ppmv control	36.6	2429 (32)	2365 (49)	7.90 (0.1)	1.8 (0.6)

Seawater Type	Salinity	T _A	T _C	pH _T	Ω _{calcite}
		(μMol kg SW ⁻¹)			
1000-ppmv incubator	36.6	2424 (56)	2376 (58)	7.8 (0.0)	1.6 (0.2)
1000-ppmv control	36.6	2419 (4)	2327 (20)	7.9 (0.0)	2.0 (0.2)
2000-ppmv incubator	36.6	2485 (31)	2535 (28)	7.5 (0.0)	0.8 (0.0)
2000-ppmv control	36.6	2422 (20)	2328 (28)	7.9 (0.1)	2.0 (0.4)

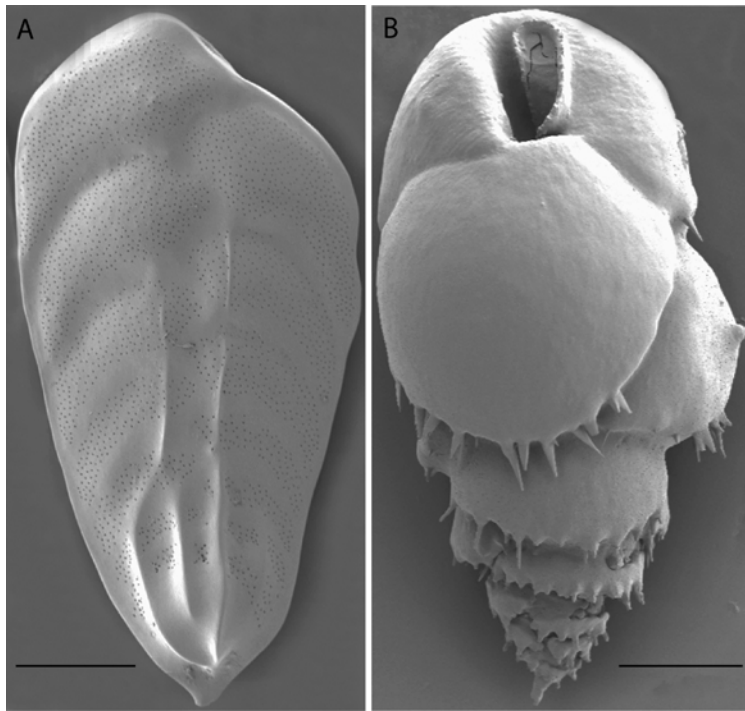


Figure 1.

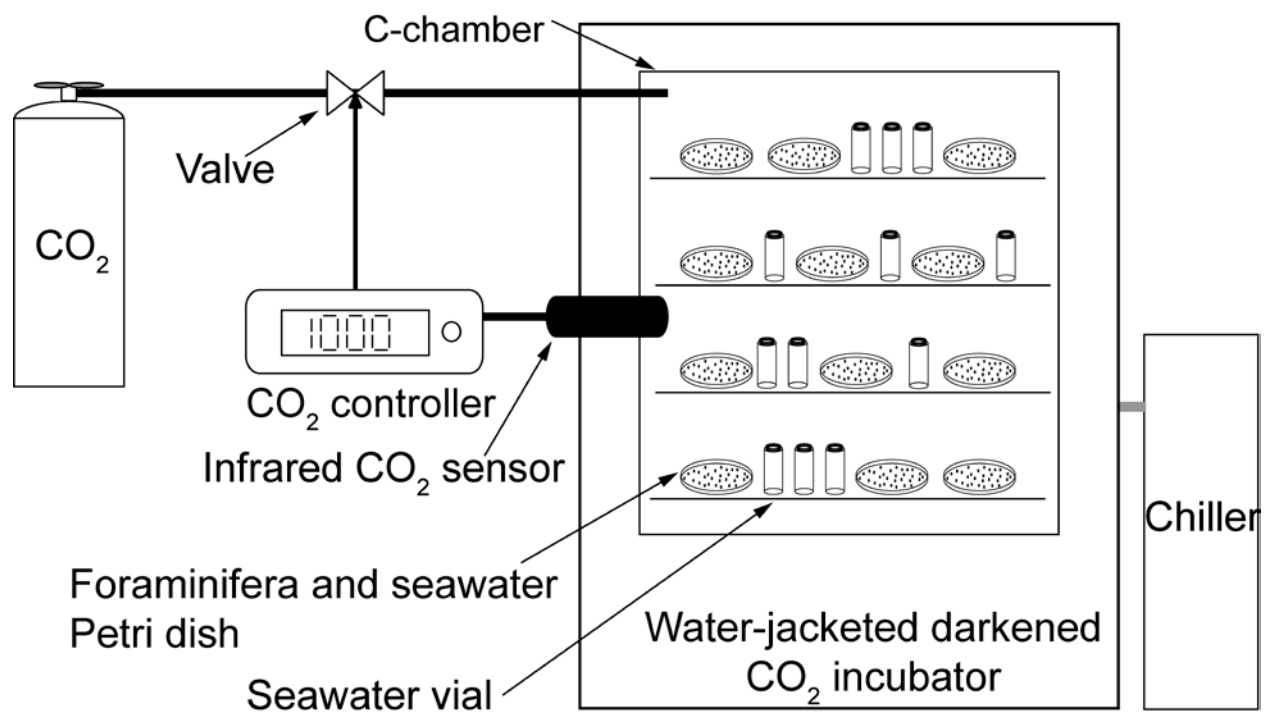


Figure 2.

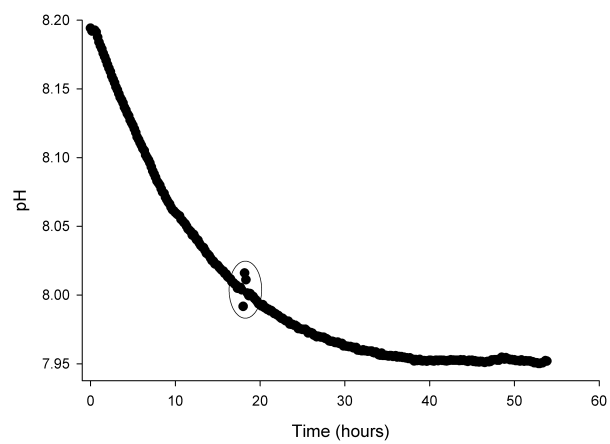


Figure 3.

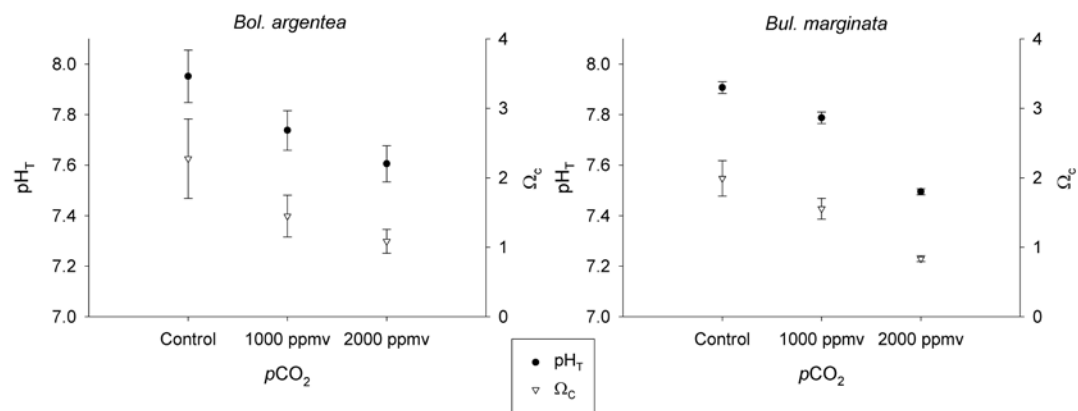


Figure 4.

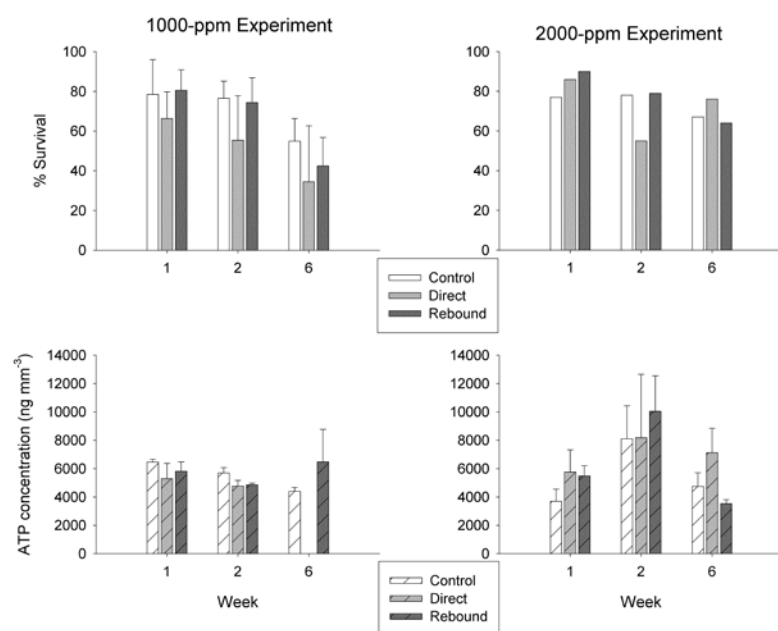
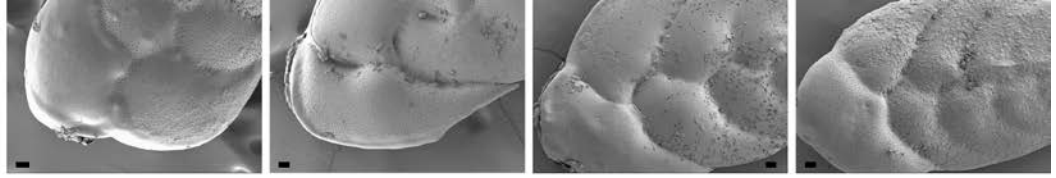


Figure 5.

Control



2000-ppm $p\text{CO}_2$

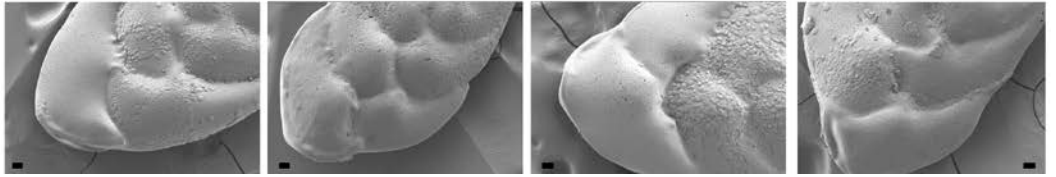


Figure 6.

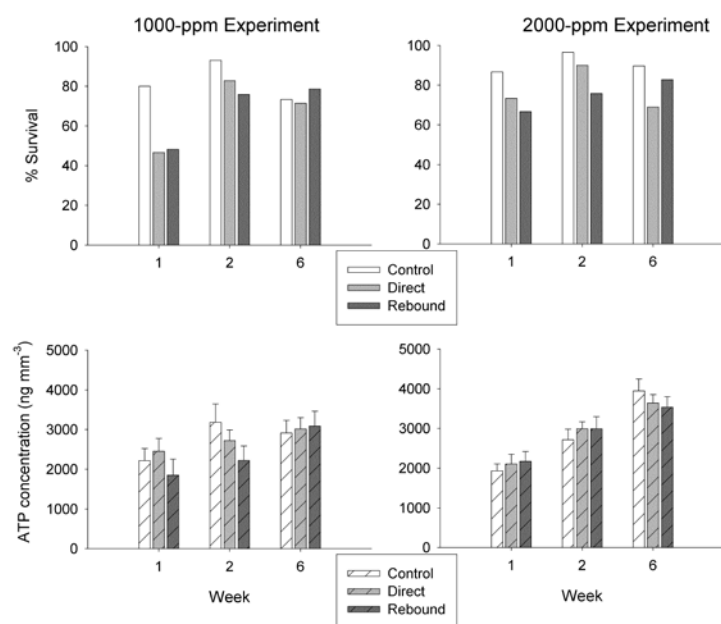
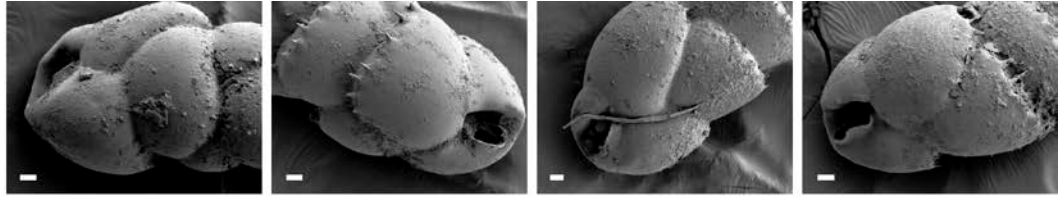


Figure 7.

Control



2000-ppm $p\text{CO}_2$

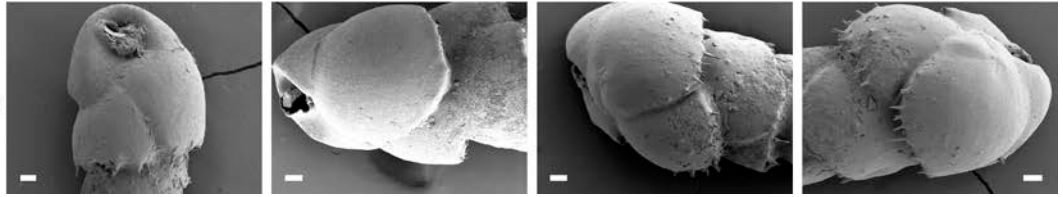


Figure 8.